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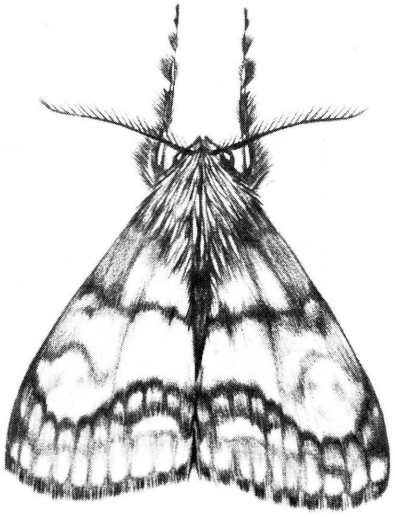
Douglas-Fir Tussock Moth Handbook

How to Determine the Occurrence of Virus in Egg Masses



How to Determine the Occurrence of Virus in Egg Masses

by M. J. Stelzer[1]

In 1974 the U.S. Department of Agriculture initiated the Combined Forest Pest Research and Development Program, an interagency effort that concentrated on the Douglas-fir tussock moth in the West, on the southern pine beetle in the South, and on the gypsy moth in the Northeast. The work reported in this publication was funded in whole or in part by the Program. This manual is one in a series on the Douglas-fir tussock moth.

The natural occurrence of a disease caused by a nucleopolyhedrosis virus (NPV) associated with the egg stage of the Douglas-fir tussock moth can have a significant impact on population survival and tree damage during outbreaks of this insect pest in western forests.

Before artificial control measures (chemical or microbial sprays) are used to control an outbreak, the mortality rate of hatching tussock moth larvae caused by the natural virus should be determined. In some areas, the effect of the natural virus is sufficient to initiate an epizootic or natural control of the tussock moth population.

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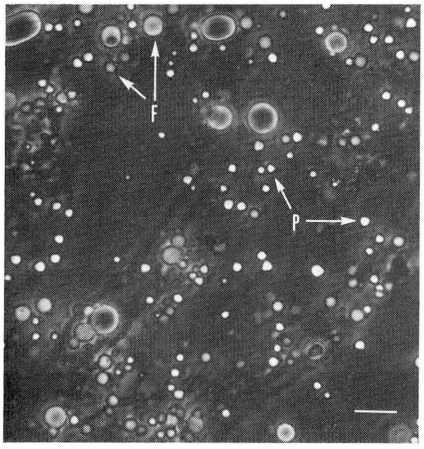

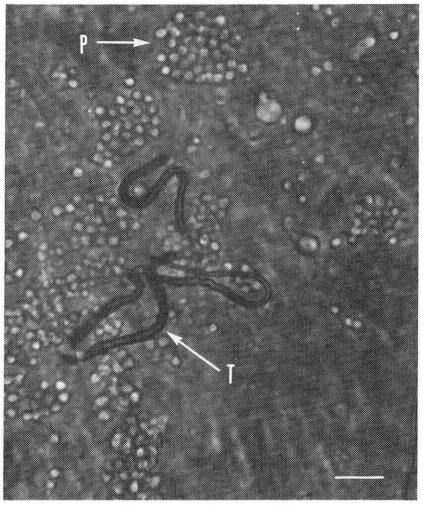

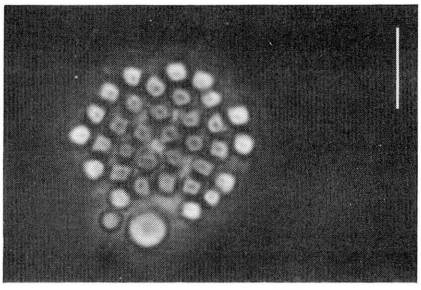

Virus-infected tussock moth larvae can be identified by the presence of polyhedra or proteinaceous inclusion bodies (fig. 1). Infection occurs when the polyhedra enter the insect gut with food material. Alkaline gut juices dissolve the polyhedra releasing infectious virus particles (virions) that invade the nuclei of susceptible tissues (fat body, hypodermis, tracheal matrix) (fig. 2). Virus particles multiply and form inclusion bodies inside infected nuclei (fig. 3) eventually causing the cells to burst and fill the larva with a whitish fluid laden with polyhedra. A large tussock moth larva can produce more than 500 million polyhedra.

Figure 1.—Insect body fluids containing numerous polyhedra indicating infection by the Douglas-fir tussock moth virus. Note the sharply angled margins of the polyhedra (P) as contrasted to the round margins of fat globules (F).
Figure 2.—Infected issue containing clusters of polyhedra (P) enclosed in the nuclear membrane. This phenomena is often seen in fat and tracheal (T) tissues at early stages of virus infection.
Figure 3.—An isolated cluster of polyhedra retained within a nuclear membrane. Bar lengths in all figures are 10 microns.

The virus disease affects the tussock moth at all stages of larval and pupal development. Following death, the diseased larvae are usually found hanging from a needle or branch with the body contents liquified. The fragile, virus-laden larvae rupture and contaminate adjacent foliage, which is then consumed by, and infects, other larvae. Egg masses are probably contaminated with virus by rain and melting snow which has been in contact with diseased pupae. The first-instar larvae are infected when they consume a portion of the contaminated shell of their own egg while hatching. Infected larvae usually die 7–14 days after hatch—before reaching the second instar.

A potentially destructive tussock moth population may not require direct control measures when the natural virus is present at or above a certain level. Although the tussock moth virus is highly contagious, the spread of the disease is slow during the first four or five instars when initial (first instar) infection rates are low (below 15 percent). Spread of the disease increases dramatically as the larvae reach maturity and begin to pupate. In situations where the incidence of virus is low during early instars, natural control of the outbreak occurs too late to prevent severe tree damage and some mortality.

In experiments, both insect population control and foliage protection have been obtained by aerial applications of virus to second- and third-instar larvae when the treatment resulted in the infection of 25 percent of the larvae. Thus it appears that tussock moth populations in which at least 25 percent of the larvae are contaminated with virus at hatch will collapse from an epizootic before unacceptable tree damage has occurred. This projection should be valid in areas where first- and second-instar larval densities exceed 100 larvae per 1,000 in^2 of branch area (Mason 1970). Additional data is still needed on the relationship of egg-virus levels to population dynamics and feeding damage—especially for lower population densities.

Collecting Egg Masses

Virus incidence can be determined from egg masses collected from infested forest stands in late fall (October-November) after the period of oviposition, or the following spring when the areas become accessible and before larvae begin to hatch (April–early May). Collections made in the spring may provide a more accurate estimate of virus infection than fall collections because of the distribution of virus by winter rain and snow.

To Collect Egg Masses

1. Search the lower branches and trunks of host trees for new egg masses. Take a few masses from many trees rather than many masses from a few trees. A sample of around 50 egg masses per stand or area is sufficient.

2. Collect egg masses individually and store them in containers such as small plastic sandwich bags. Use sterile toothpicks or forceps to dislodge the egg masses and drop them into the bags. Sterilize equipment such as forceps after each egg mass by dipping in a 2-percent solution (in water) of common household bleach. Toothpicks are readily disposable and should be discarded following collection of each mass.

3. Keep collected egg masses out of direct sunlight and in a cool place while they are being transported.

Determining Virus Presence

Egg masses collected in the fall must be refrigerated to satisfy diapause requirements before the larvae will hatch. Generally, when eggs are stored at 5° C for 4 months, optimal hatch results. Eclosion or larval hatch will start about 14 days after the eggs are brought to room temperature (25° C). Egg masses collected in the spring do not require refrigeration to hatch. When hatch begins will depend on how close the collection date was to the natural hatching date. Because the disease is highly contagious, work areas should be kept clean and wiped periodically with disinfectant.

To Determine Presence of Virus

1. Remove egg masses from the refrigerator and transfer them to 15 × 100-mm petri dishes, one mass per dish.

2. Maintain the egg masses at 25° C and about 40-50 percent relative humidity with alternating 12-hour periods of light and dark. Larval hatch will usually begin in 12-14 days.

3. Remove the egg masses from the petri dishes, leaving 25 larvae that have hatched within a 1- to 3-day period.

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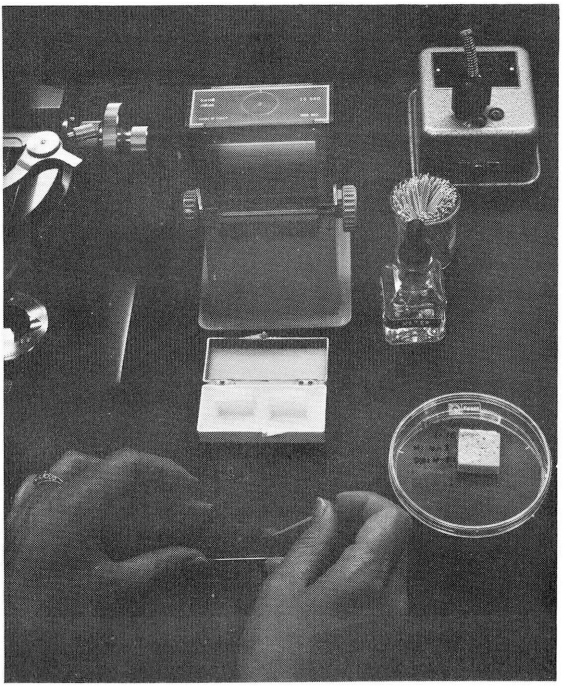

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Egg mass No.	No. larvae reared		Mass incidence (%)
	Total	Killed by virus	
1	25	4	16.0
2	25	0	0
3	25	12	48.0
4	25	9	36.0
etc.	etc.	etc.	etc.
N			
Totals			$\bar{X}$ =

Figure 4.—Equipment and materials used in the preparation of dead tussock moth larvae for diagnosis.
Figure 5.—Sample form for recording virus incidence data.

4. Add a square (2 × 2 cm) of tussock moth diet to each petri dish. Prepare diet according to instructions of Thompson and Peterson (1978).

5. Refeed the larvae after 7 days, removing the old diet when a new supply is added.

6. Stop rearing after 14 days and examine the dead larvae for the presence of virus. It is important that the rearing period is terminated at 14 days after hatch to minimize infection and mortality due to contagion.

To Determine Virus Mortality

1. Place the dead larva, or a portion of the larva, on a microscope slide in a drop of sterile distilled water (fig. 4).

2. Apply a coverslip, with pressure if necessary, to rupture the integument and disperse the body contents.

3. Examine this material using bright-field or phase-contrast light microscopy at a magnification of 400× or higher (figs. 1, 2, and 3).

4. Calculate the percent of virus mortality of each mass and determine the average for all masses from the same sample area or stand (fig. 5).

References

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